chemist the ability to achieve the desired selectivity in a single, convenient extraction procedure.

Due to the additional parameters of temperature and pressure that modern instrumental techniques provide, it has become more difficult to compartmentalize extraction techniques based on whether the extraction fluid is a dense gas, liquid, supercritical fluid, or combination thereof. Strictly speaking, pressurized fluid extraction PFE includes all types of pressurized extractions independent of the solvent's state of matter. Subcategories of PFE include SFE and PLE, but instrument companies have confused the terminology by marketing PLE as accelerated solvent extraction (ASE^{TM}) and enhanced solvent extraction (ESE). Other scientists have developed other terms to describe extraction techniques, such as enhanced fluidity extraction, which connotes a mixture of gas, liquid and/or supercritical fluid, and subcritical water extraction, which is meant to represent PLE using water at high temperatures. However, the unifying principles of extraction are the same no matter what instrument-makers or scientists may call a particular approach.

See also: **II/Chromatography: Gas:** Headspace Gas Chromatography. **Extraction:** Solid-Phase Extraction; Solid-Phase Microextraction; Solvent Based Separation; Supercritical Fluid Extraction. **III/Environmental Applications:** Soxhlet Extraction; **Microwave-Assisted Extraction: Environmental Applications. Solid-Phase Matrix Dispersion: Extraction.**

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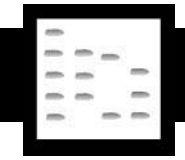
NATURAL PRODUCTS



High-Speed Countercurrent Chromatography

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In high speed countercurrent chromatography (HSCCC) a sample is partitioned between two nonmiscible liquid phases. One phase is held stationary by a centrifugal force (applied by spinning the separation element at high speed), while the second phase is pumped through the apparatus, hence the alternative term for the process centrifugal partition chromatography (CPC).

Unlike high performance liquid chromatography (HPLC), in which the stationary phase occupies 5–7% and the mobile phase about 75% of the column, the relative proportions in HSCCC are 50–75% for the stationary phase and 20–50% for the mobile phase. As a consequence, large sample loads are possible with HSCCC. Another important advantage of the absence of a solid support is that irreversible adsorption is avoided. There is total recovery of the injected sample and tailing is minimized. HSCCC is thus of special importance for the separation of sensitive and easily degraded samples. Although the efficiency of HSCCC separations is lower than that encountered in HPLC, the optimization of selectivity is the great advantage offered by the former technique.

The potential of HSCCC is further shown by the possibility of applying gradients for separations.

Solvent proportions can be changed during a chromatographic run. Furthermore, solvent elution can be reversed in the course of a separation by changing over stationary and mobile phases. Consequently, one of the characteristics of HSCCC is its extreme flexibility.

Most applications of HSCCC have been performed on two types of instrument. The first category, namely rotating coil instruments, consists of a polytetrafluoroethylene tube (column) wrapped around a spool. The spool is rotated around a central axis in such a way that it describes a planetary motion. Alternatively, hydrostatic equilibrium instruments can be employed. These have either cartridges or discs arranged around a central axis. The separation column, in effect, consists of a series of cells in the cartridges or discs.

Preparative Applications

HSCCC is becoming a routine preparative technique in both industrial and university laboratories. Sample sizes ranging from milligrams to grams (and even kilograms in specialized instruments) can be successfully chromatographed. An extensive range of separations by HSCCC has been published (see Further Reading).

Aqueous and nonaqueous solvent systems are used and the separation of compounds with a wide range of polarities is possible. Two-phase solvents are chosen according to the hydrophobicity of the sample. For polar compounds, *n*-butanol can be employed (e.g. ethyl acetate–butanol–water or chloroform–butanol–water systems), for moderately hydrophobic compounds chloroform (e.g. chloroform–methanol–water) solvent systems, and for more hydrophobic compounds, *n*-hexane (e.g. *n*-hexane–ethyl acetate–methanol–water) solvent systems. Some of the more frequently used solvent systems are shown in **Table 1**.

A number of representative separations by HSCCC are presented here, in order to give an idea of the possibilities available, together with the chromatographic conditions employed.

Plant-derived Natural Products

Flavonoids Many HSCCC separations of natural products involve polyphenols. The reason for this fact is that there is a tendency to 'tail' or even to adsorb irreversibly on conventional chromatographic supports (silica gel, polyamide etc.). This problem does not occur with all-liquid separation techniques and quantitative recovery of injected sample is achieved.

Separations of flavonoid aglycones and flavonoid glycosides have been performed mainly with chloroform–methanol–water or chloroform–methanol–propanol–water systems.

Table 1 Frequently used solvent systems for HSCCC

Sample	*Solvent system*
Polar	$CHCl_3$–MeOH–*n*-BuOH–H_2O 7 : 6 : 3 : 4
	n-BuOH–*n*-PrOH–H_2O 4 : 1 : 5
	n-BuOH–*n*-PrOH–H_2O 2 : 1 : 3
	EtOAc–*n*-BuOH–H_2O 3 : 2 : 5
Moderately polar	$CHCl_3$–MeOH–H_2O 4 : 3 : 2
	$CHCl_3$–MeOH–H_2O 5 : 6 : 4
	$CHCl_3$–MeOH–H_2O 10 : 10 : 6
	$CHCl_3$–MeOH–H_2O 13 : 7 : 8
	$CHCl_3$–MeOH–H_2O 7 : 13 : 8
	n-Hexane–EtOAc–MeOH–H_2O 1 : 1 : 1 : 1
Apolar	*n*-Hexane–EtOAc–MeOH–H_2O 10 : 5 : 5 : 1
	n-Hexane–CH_3CN–MeOH 8 : 5 : 2
	n-Hexane–CH_3CN–CH_2Cl_2 10 : 7 : 3

The time required for a particular separation can be reduced by incorporating reversed-phase (RP) operation into the procedure. **Figure 1** shows the situation for the flavanone hesperitin (**1**) and the flavonols kaempferol (**2**) and quercetin (**3**). When eluting with the upper phase (mobile phase), the most polar component eluted first and the time for complete separation is over 8 h. Reversing the elution mode and changing to the lower phase as mobile phase at 70 min leads to completion of the separation in just over 2 h.

Alternatively, gradient operation can be used to speed up HSCCC. By pumping simultaneously the upper and lower phases of the two-phase solvent system by separate pumps, the proportions of the phases are changed in the coil during separation. In the example shown in **Figure 2**, the coil (capacity 360 mL) of the chromatograph was initially filled with equivalent amounts of upper and lower phases of the solvent used in Figure 1. The flavonoid mixture was then injected. By pumping upper phase at 4 mL min^{-1} and lower phase at 1 mL min^{-1} through the apparatus, the content of the lower phase in the coil was increased from 180 to 340 mL over 3 h. The separation time of the three flavonoids was thus reduced by 5 h, when compared with normal operation.

Xanthones This is another class of polyphenols to which liquid–liquid chromatography has successfully been applied. Extensive use of HSCCC was made for the separation of xanthones from a plant, *Hypericum roeperanum* (Guttiferae), from Zimbabwe. A dichloromethane extract of the roots gave a fraction (363 mg) rich in xanthones after initial chromatography. Separation of the individual xanthones from this

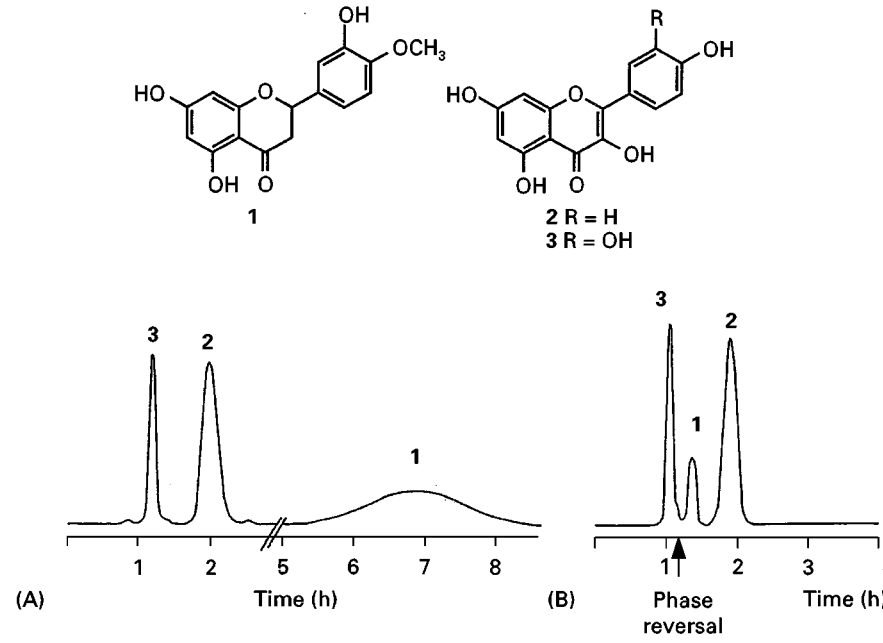


Figure 1 HSCCC separation of hesperetin (**1**), kaempferol (**2**) and quercetin (**3**) by normal and reversed-phase solvent elution. Instrument: multilayer countercurrent chromatograph (PC Inc.). Solvent system: $CHCl_3$–MeOH–H_2O 5 : 6 : 4. Detection: 254 nm. (A) Upper phase as mobile phase; flow rate 3 mL min^{-1}; rotational speed 700 rpm. (B) Upper phase as mobile phase to 70 min, then lower phase as mobile phase; flow rate 3 mL min^{-1}; rotational speed 700 rpm. (Reproduced with permission from Marston A, Slacanin I and Hostettmann K (1990) Centrifugal partition chromatography in the separation of natural products. *Phytochemical Analysis* 1: 3–17. Copyright 1990, John Wiley and Sons Limited.)

fraction by other methods, including semipreparative HPLC, proved very difficult. However, HSCCC gave six fractions which, after final purification steps provided eight xanthones. For the HSCCC separation, the solvent hexane–EtOAc–MeOH–H_2O 1 : 1 : 1 : 1 was employed, with the upper phase as the mobile phase. One of the new xanthones isolated, 5-*O*-dimethyl-paxanthonin (**4**), could only be satisfactorily separated by HSCCC.

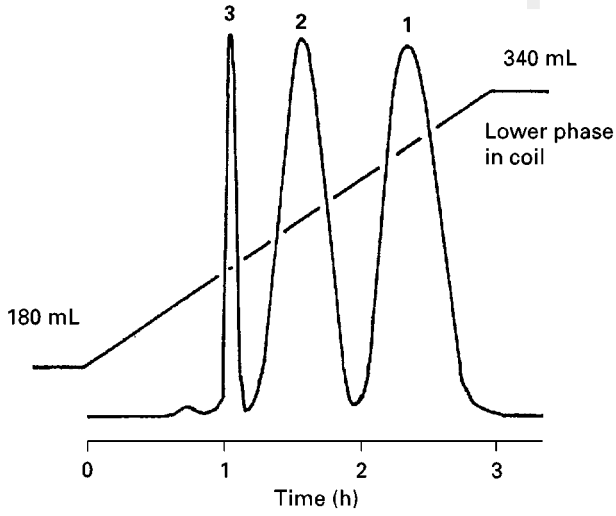
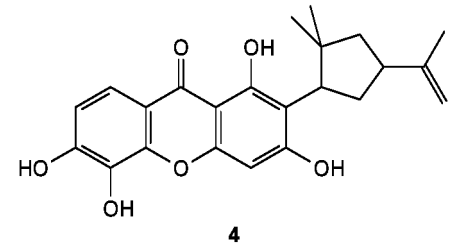
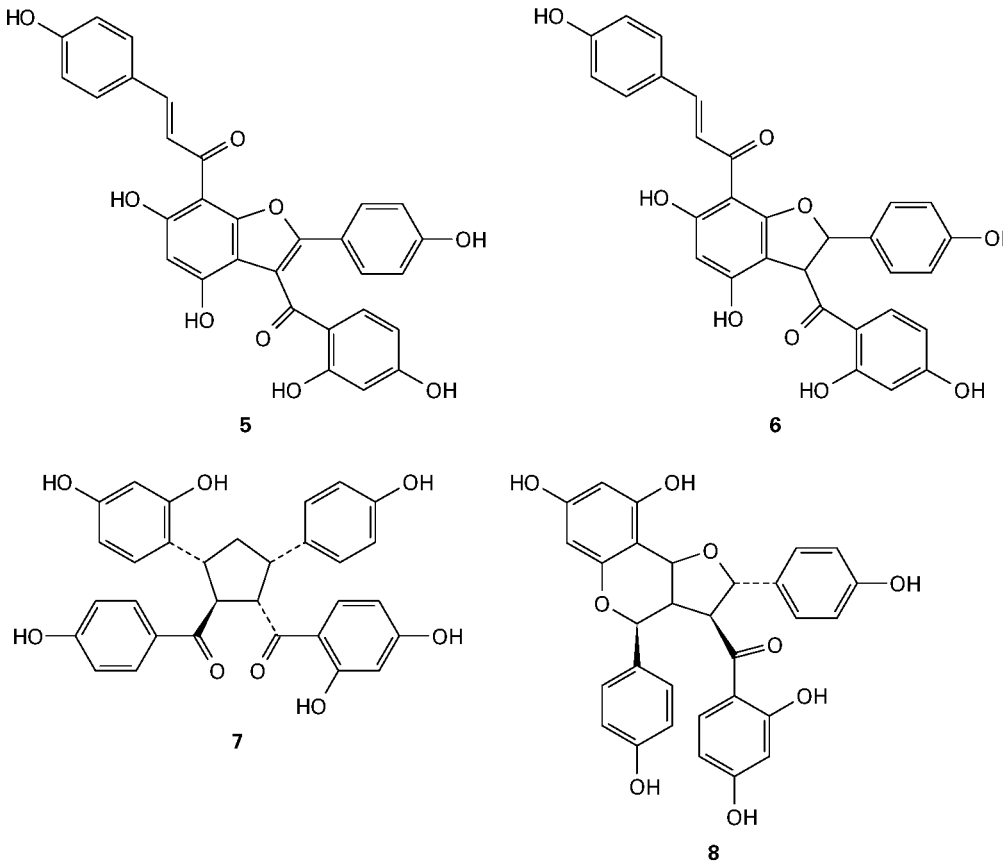


Figure 2 Gradient elution for the separation of flavonoids **1–3**. Instrument: multilayer countercurrent chromatograph (PC Inc.). Solvent system: $CHCl_3$–MeOH–H_2O 5 : 6 : 4. Composition of mobile phase: upper phase at 4 mL min^{-1}, lower phase at 1 mL min^{-1}. (Reproduced with permission from Slacanin I, Marston A and Hostettmann K. (1989) Modifications to a high-speed countercurrent chromatograph for improved separation capability. *Journal of Chromatography* 482: 234–239. Copyright 1989, Elsevier Science.)



Chalcone derivatives A further example of the separation of polyphenols by HSCCC is provided by the isolation of chalcone derivatives **5–9** from *Brackenridgea zanguebarica*, a tree found in central Africa and belonging to the Ochnaceae family. This application of HSCCC also illustrates that large amounts of crude plant extract can potentially be handled.

Direct fractionation of 20 g of a methanol extract of the yellow bark layer from the tree is possible with the solvent system cyclohexane–ethyl acetate–methanol–water 8 : 8 : 6 : 6 (upper layer as mobile phase). The extract was dissolved in 20 mL of each phase and



introduced via a 60 mL sample loop. The separation column (660 mL) was first filled with 50% of each phase and then eluted with mobile phase. When steady run conditions had been achieved, the sample was injected. Seven fractions were obtained (A–G; **Figure 3**), three of these giving the antifungal polyphenols **5–8**. Compound **5** crystallized from fraction B, while compounds **6–8** were purified by a final low pressure liquid chromatography step on a C_{18} support. A novel, inactive, spiro derivative (**9**) was isolated from fraction E after low pressure liquid chromatography on a C_{18} stationary phase.

As can be seen, no more than two separation steps were required for each polyphenol isolated.

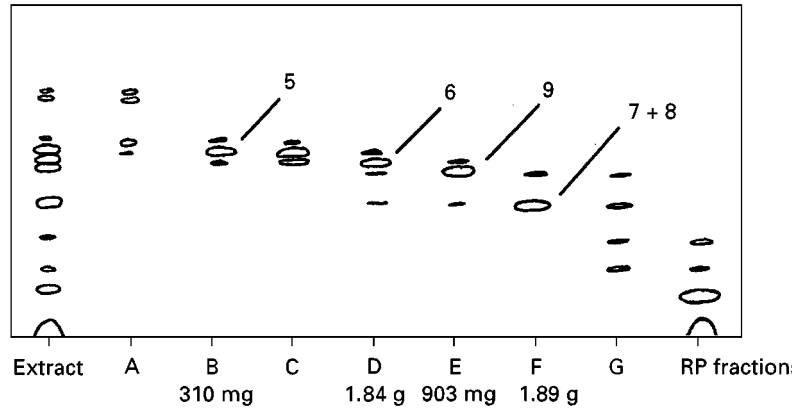
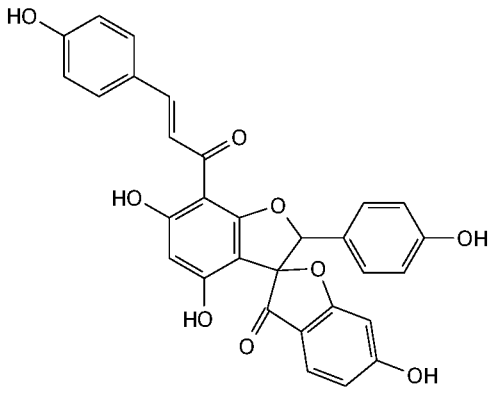


Figure 3 Thin-layer chromatography monitoring (solvent: lower phase of $CHCl_3$–MeOH–H_2O 2 : 2 : 1) of fractions from the CCC-1000 HSCCC separation of *Brackenridgea zanguebarica* stem bark methanol extract. Solvent system: cyclohexane–EtOAc–MeOH–H_2O 8 : 8 : 6 : 6 (upper phase as mobile phase). Flow rate: 3 mL min^{-1}. Rotational speed: 1000 rpm. Detection: 254 nm. Sample size: 20 g.



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Phenylpropanoids and coumarins It is possible to isolate natural products by HSCCC alone, as exemplified by the purification of phenylpropanoids and a furanocoumarin from a dichloromethane extract of the leaves of *Diplolophium buchanani* (Apiaceae). Initial fractionation gave semipure products (**Figure 4**). A subsequent liquid–liquid step, using a different nonaqueous solvent system in each case, gave pure myristicin (**10**, using hexane–*t*-butyl methyl ether–acetonitrile 5 : 1 : 5; upper layer as mobile phase) and a mixture of elemicin (**11**) and *trans*-isoelemicin (**12**) (using heptane–acetonitrile–methanol 6 : 3 : 1; upper layer as mobile phase). The furanocoumarin oxypeucedanin (**13**) was obtained by simple crystallization of the corresponding HSCCC fraction. All four isolated compounds had both antifungal and larvicidal activities.

Lignans Initial fractionation of insecticidal neolignans from *Magnolia virginiana* (Magnoliaceae) by HSCCC was better, less expensive and more efficient than traditional open-column or more recent flash chromatographic methods. A hexane extract of the leaves was chromatographed with the lower layer of the solvent system hexane–acetonitrile–ethyl acetate–water 8 : 7 : 5 : 1 as mobile phase. This solvent contained only a small proportion of water to provide compatibility with the very lipophilic extract. Subsequent purification of the fractions provided a biphenyl ether (**14**) and two biphenyls (**15**, **16**), which were not only insecticidal to *Aedes aegypti* (the vector of yellow fever) but also fungicidal, bactericidal and toxic to brine shrimp.

During the investigation of anti-human immunodeficiency virus type 1 lignans from creosote bush, *Larrea tridentata* (Zygophyllaceae), a cross-axis coil planet centrifuge was employed with aqueous phases containing sodium chloride, e.g. hexane–ethyl acetate–methanol–0.5% NaCl 6 : 4 : 5 : 5. The presence of salt reduced emulsification and gave improved stationary-phase retention.

Tannins Tannins include a wide variety of phenolic compounds, ranging from single glycosides of gallic

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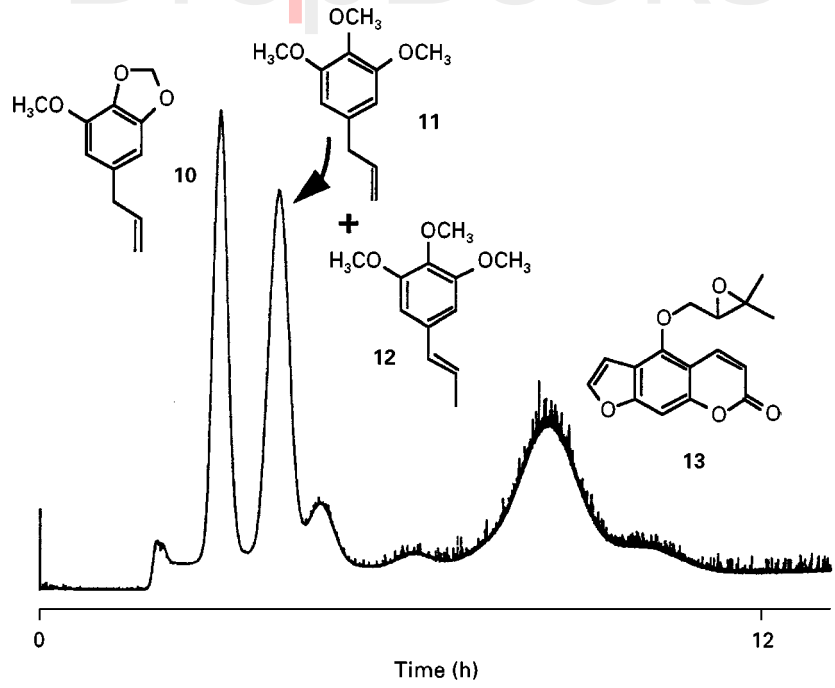
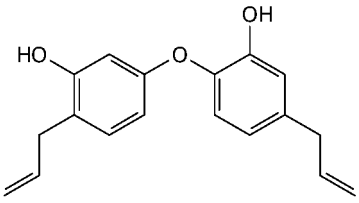


Figure 4 HSCCC initial fractionation on a CCC-1000 instrument of a dichloromethane extract of *Diplolophium buchanani* leaves. Solvent system: hexane–EtOAc–MeOH–H_2O 10 : 5 : 5 : 1 (upper phase as mobile phase). Flow rate 3 mL min^{-1}. Rotational speed: 1000 rpm. Detection: 254 nm. Sample size: 1.7 g.



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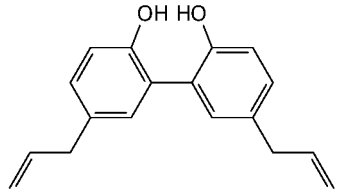

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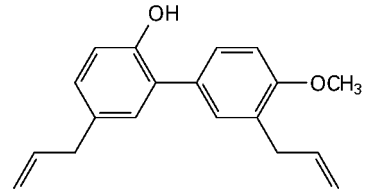

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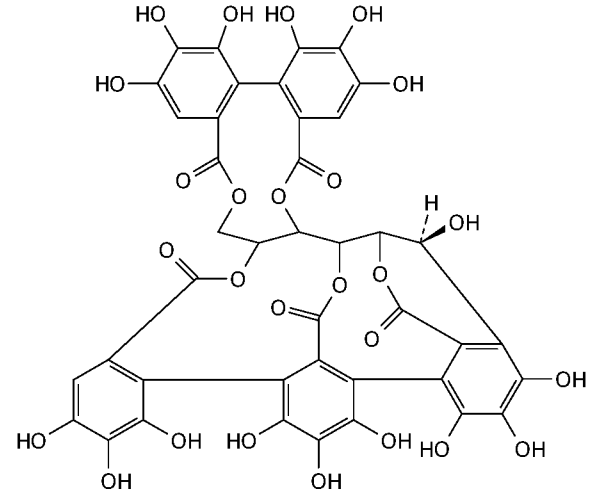
acid to complex condensed and polymerized derivatives of catechin, epicatechin and related compounds. Their separation poses special problems since there is often irreversible adsorption and even hydrolysis on solid supports. Preparative HPLC is accompanied by sample loss and deterioration or contamination of the column. HSCCC has proved to be an ideal technique for the resolution of these particular problems.

Among the examples of successful separations performed on cartridge instruments is the resolution of two diastereomers, castalgin (**17**) and vescalagin (**18**), which differ only in the configuration of a single hydroxyl group. They were extacted from *Lythrum anceps* (Lythraceae) leaves and chromatographed with the solvent system *n*-butanol–*n*-propanol–water (4 : 1 : 5), using the upper phase as the mobile phase.

The same solvent system was used to obtain a trimeric (nobotanin J; molecular weight 2764) and a tetrameric (nobotanin K; molecular weight 3742) hydrolysable tannin from the leaves of *Heterocentron roseum* (Melastomataceae). The isolation procedure involved chromatography on Toyopearl HW-40 (methanol–acetone–water gradient). Final purification of the two tannins was on an instrument with 12 cartridges (total capacity 240 mL; 700 rpm; flow rate 3 mL min^{-1}).

Monoterpene glycosides Among the examples of monoterpene glycosides that have been separated by HSCCC are two secoiridoid glycosides from the South American plant *Halenia campanulata* (Gentianaceae). Size exclusion chromatography of a crude methanol extract of the plant on Sephadex LH-20, followed by separation by HSCCC (1000 rpm) with the solvent system $CHCl_3$–MeOH–H_2O 9 : 12 : 8 (lower phase as mobile phase; flow rate 3 mL min^{-1}) gave the two glycosides (**19**, **20**). Their separation is possible by HSCCC, despite the fact that they only differ in their configuration at C_7.

Triterpene glycosides Liquid–liquid chromatography is particularly suitable for the separation of highly polar compounds. Triterpene glycosides enter into this category and many successful isolations have



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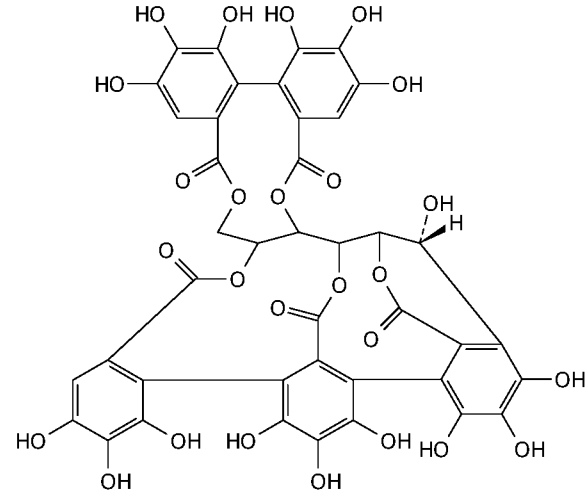

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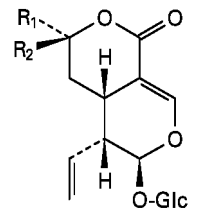

19 $R_1 = OCH_3$, $R_2 = H$
20 $R_1 = H$, $R_2 = OCH_3$

been performed using HSCCC either alone or in combination with other chromatographic methods. The direct separation of pure saponins from the crude methanol extract of *Hedera helix* (Araliaceae) berries has proved possible by this method. The extract was first partitioned between *n*-butanol and water. The *n*-butanol fraction was subjected to HSCCC, eluting with the lower layer of the solvent system $CHCl_3$–MeOH–H_2O 7 : 13 : 8. Elution was monitored by thin-layer chromatography and gravimetry. Molluscicidal saponins **21–24**, with hederagenin as aglycone, were separated within 2 h by this technique (**Figure 5**).

Alkaloids The utility of CPC in separating alkaloids from gummy or tarry matrices has been shown in the preparative separation of pyrrolizidine alkaloids from different plant sources. After classical alkaloid extraction procedures, batches of up to 800 mg of extract could be chromatographed on a rotating coil apparatus (380 mL capacity). Potassium phosphate buffer (0.2 mol L^{-1}) at an appropriate pH was used as stationary phase, while the mobile phase was chloroform. With the buffered stationary phase, good solute resolution was achieved since structurally similar pyrrolizidine alkaloids differ in their pKa values.

HSCCC has been employed for the separation of the antitumour drug, camptothecin (**25**). From sources such as *Nothapodytes foetida* (Icacinaceae), camptothecin is often mixed with 9-methoxycamptothecin (**26**). These can be easily separated with the solvent systems $CHCl_3$–CCl_4–MeOH–H_2O 2 : 2 : 3 : 1 or CH_2Cl_2–MeOH–H_2O 5 : 3 : 1. The low solubility of the samples poses a problem with traditional chromatographic techniques. However, for the HSCCC separation, 500 mg of sample can be dissolved

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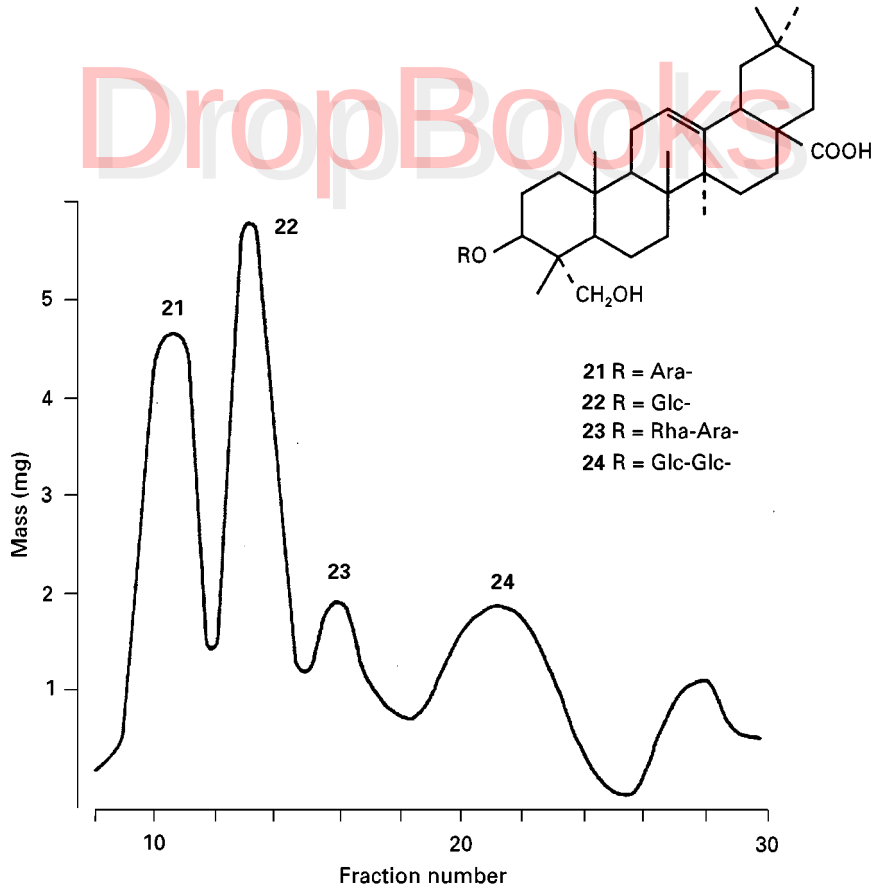
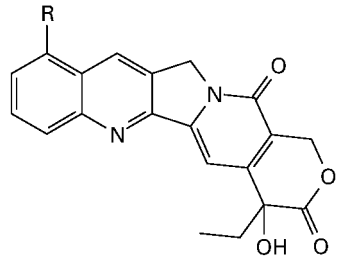


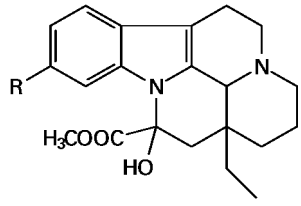
Figure 5 Separation of a methanol extract of *Hedera helix* berries on a Sanki CPC instrument. Solvent system: $CHCl_3$–MeOH–H_2O 7 : 13 : 8 (lower phase as mobile phase). Flow rate 1.5 mL min^{-1}. Rotational speed: 700 rpm. Sample size: 100 mg.

in 70 mL lower phase and 10 mL upper phase (i.e. a large volume) for injection via a sample loop.



25 R = H (Camptothecin)
26 R = OCH_3 (9-Methoxycamptothecin)

Repetitive sample injections are possible for the separation of close-running compounds on rotating coil instruments. This has been shown for the separation of vincamine (**27**) and vincine (**28**) from *Vinca minor* (Apocynaceae). After 20 successive injections (at 42 min intervals), each of 1.7 mg sample mixture, 16.5 mg of **27** and 14 mg of **28** were obtained on a 230 mL rotating coil instrument. The solvent system was *n*-hexane–ethanol–water (6 : 5 : 5; lower phase as mobile phase). The resolution of the HSCCC system was not changed when the instrument was shut down overnight and restarted the next day with the same stationary phase in the column.



27 R = H (Vincamine)
28 R = OCH_3 (Vincine)

Marine Natural Products

The mild conditions achieved with HSCCC and the rapidity of the method are ideal for the separation of delicate marine natural products. Attempts at purification of antitumour ecteinascidins from the tunicate *Ecteinascidia turbinata*, for example, by normal-phase or RP chromatography leads to extensive loss of activity. HSCCC, however, proved to be an effective means of separating these light- and acid-sensitive alkaloids.

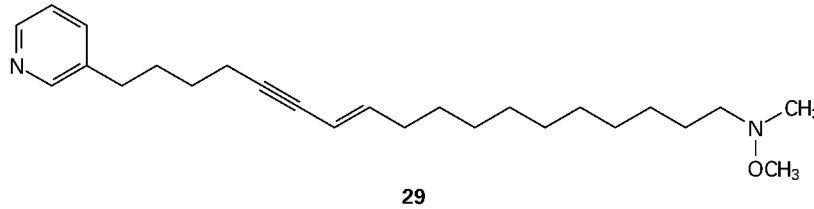
The pyrroloquinoline alkaloids isobatzellines A–C have been isolated from a *Batzella* sponge. They exhibited *in vitro* cytotoxicity against the P-388 leukaemia cell line and antifungal activity against *Candida albicans*. HSCCC with a rotating coil apparatus was used in their purification, following extraction and solvent partitioning. Elution was performed with the upper phase of the solvent system heptane–chloroform–methanol–water (2 : 7 : 6 : 3).

The nonaqueous solvent system heptane (or hexane)–dichloromethane–acetonitrile (10 : 3 : 7) has been applied to the separation of a variety of marine natural products, including a long chain methoxylamine pyridine, xestamine A (**29**), isolated from the sponge *Xestospongia wiedenmayeri*.

Antibiotics

Since its inception, HSCCC has been associated with the field of antibiotics. Liquid–liquid partition techniques are particularly suitable for their separation because bioactive metabolites are often produced in small amounts and have to be removed from other secondary metabolites and nonmetabolized media ingredients. Antibiotics are normally biosynthesized as mixtures of closely related congeners and many are labile molecules, thus requiring mild separation techniques with a high resolution.

To illustrate the applications of HSCCC to the separation of antibiotics, one example is provided by the sporavidins, which are water-soluble basic glycoside antibiotics with complex structures. These are unstable under basic conditions and exist as mixtures of closely related compounds. A sample comprising six sporavidins was resolved on a rotating coil countercurrent instrument (total capacity 325 mL; 800 rpm). Selection of the solvent system was based on partition coefficient data from chloroform–methanol–water, chloroform–ethanol–methanol–water and *n*-butanol–diethyl ether–water mixtures. After HPLC analysis, the final system adopted was *n*-butanol–diethyl ether–water (5 : 2 : 6). Sample was introduced by the so-called sandwich technique, in which sample was injected after filling with stationary phase and before mobile-phase elution. The six components were separated within 3.5 h, employing a total elution volume of 500 mL.



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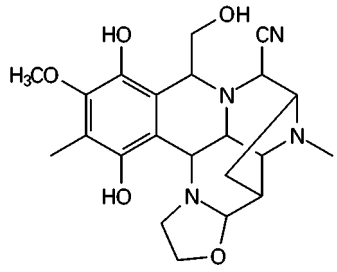
Streptomyces lusitanus produces the hydroquinone derivative **30** (cyanocycline C), which is extremely unstable and cannot be purified by semipreparative HPLC. However, isolation of this antibacterial compound was successfully performed on a rotating coil apparatus with the solvent system $CHCl_3$–MeOH–iPrOH–H_2O 3 : 10 : 10 : 10.



30

Analytical Applications

Reducing the radius of revolution of a rotating coil and increasing the speed of rotation have produced highly efficient HSCCC systems with an effective analytical capability. Operational speeds lie between 2000 and 4000 rpm and the tube bore is less than 1 mm i.d. Efficient separations of microquantities of samples in a short time are thus possible. In general, the observed resolution and speed are comparable to those of HPLC. However, it is unlikely that HSCCC will displace HPLC for purely analytical applications. By comparison with analytical HPLC, the major advantage of HSCCC is that it is always possible to reverse the elution mode – this is useful for complex samples with a wide range of polarity. The other important use of analytical HSCCC is in the rapid selection of suitable solvent systems for scale-up to preparative applications.

The instruments available typically have a single rotating coil, with a total capacity of 5–40 mL. Flow rates are directly related to the selected solvent system and must be adapted in order to minimize backpressure and leakage of the stationary phase. Although instruments are commercially available, many applications have been performed on prototype equipment.

While applications of analytical HSCCC to natural products are not numerous, many different classes of compounds have been separated: alkaloids, anthraquinones, coumarins, flavonoids, lignans, terpenoids and macrolides.

For example, the separation of a mixture of vincamine (**27**), the major alkaloid of *Vinca minor* (Apocynaceae) and vincine (11-methoxyvincamine) (**28**), has been performed by analytical HSCCC in hexane–ethanol–water (6 : 5 : 5) with a 0.85 mm i.d. multilayer coil. Comparison of the separation with results obtained from analytical RP-HPLC showed that both methods gave baseline resolution, but it was possible to observe a small peak just preceding the vincine peak in analytical HSCCC which was not resolved by RP-HPLC. Analysis of the sample by HSCCC-mass spectrometry showed that the minor compound was probably an isomer of vincine.

A crude flavonoid mixture obtained from the ethanolic extract of the fruits of sea buckthorn (*Hippophae rhamnoides*, Elaeaginaceae) has been successfully separated with an analytical HSCCC instrument. The separation of a 3 mg mixture with chloroform–ethanol–water (4 : 3 : 2) was complete within 15 min when the mobile lower phase was pumped at a flow rate of 5 mL min^{-1} (rotation speed 1800 rpm). The five main components of the fruit extract were resolved (including isorhamnetin, the principal flavonoid) within 8 min, a time scale wholly comparable to analytical HPLC separations. The instrument used has a 0.85 mm i.d. coil, with a 2.5 cm radius of revolution and a capacity of 8 mL.

Conclusion

The advantages of HSCCC favour a much wider use of this technique in the future: the absence of a solid chromatographic support avoids irreversible adsorption of samples; economics are favourable, as the consumption of solvent is up to 10 times less than conventional chromatographic techniques. Other features, such as the use of step and continuous gradients and the possibility of RP operation, add to the flexibility of the method. Very high sample loading is possible and large quantities of crude plant extracts can be injected without causing contamination problems. HSCCC, however, cannot be called a competitor to preparative-scale HPLC but it is rather a complementary technique. This complementarity can also be exploited when other chromatographic techniques fail to separate a given sample: HSCCC can be tried since its selectivity may be different.

It is still necessary to make improvements on the mechanical side. A variety of instruments are available but some are not yet reliable enough. Once larger scale production is underway, this sort of problem will be eliminated and the full potential of HSCCC will be realized.

See also: **II / Chromatography: Liquid:** Countercurrent Liquid. **III / Alkaloids:** Gas Chromatography; Liquid Chromatography; Thin Layer (Planar) Chromatography. **Antibiotics:** High-Speed Countercurrent Chromatography; Supercritical Fluid Chromatography. **Essential Oils:** Thin-Layer (Planar) Chromatography. **Natural Products:** High-Speed Countercurrent Chromatography;

DropBooks

Liquid Chromatography; Liquid Chromatography-Nuclear Magnetic Resonance; Supercritical Fluid Extraction; Thin Layer (Planar) Chromatography. **Terpenoids: Liquid Chromatography.**

Further Reading

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Liquid Chromatography

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Chemical investigation of plant constituents is strongly linked to the use of liquid chromatography (LC) at both the analytical and preparative level. Indeed, most of the secondary metabolites can be efficiently separated or isolated by different liquid chromatographic techniques. The plant extracts are generally screened by different bioassays and submitted to fractionation by chromatography. The fractions obtained are further tested for their biological activities. This process is repeated until the isolation of a pure active constituent, which is finally identified by spectroscopic methods (bioactivity guided isolation; **Figure 1**).

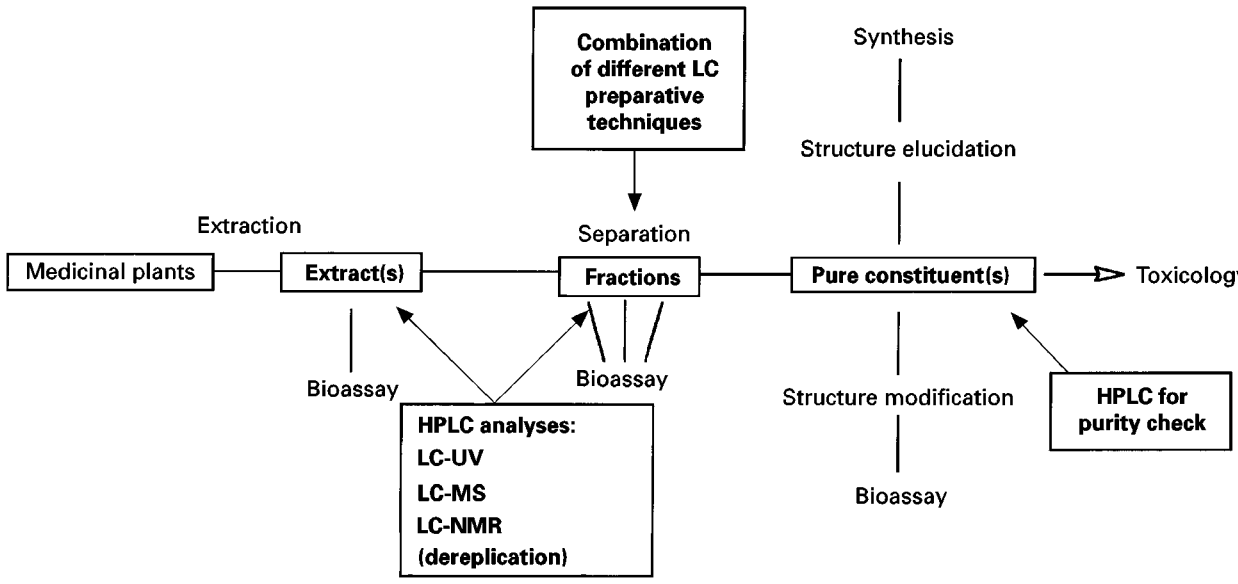

Figure 1 Procedure for obtaining the active principles from plants. LC techniques are used at the analytical and preparative level during the whole isolation procedure. HPLC hyphenated techniques play an important role in the early recognition of well-known compounds in the extract prior to isolation.